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Establishment and two-year growth of a bio-energy plantation with fast-growing *Populus* trees in Flanders (Belgium): Effects of genotype and former land use

L.S. Broeckx[*,1], *M.S. Verlinden*[1], *R. Ceulemans*

Department of Biology, Research Group of Plant and Vegetation Ecology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

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ABSTRACT

In April 2010, a large-scale Short Rotation Coppice (SRC) plantation was established with mainly poplar (*Populus* spp.) on a former agricultural site (cropland and pasture) in Flanders. The 12 selected genotypes planted were assessed on establishment and production characteristics during the first two years of growth and were found highly productive, with a volume index ranging between 1.00 (±0.68) and 1.93 (±0.97) dm^3 in growing season 1 (GS1) and between 2.75 (±1.70) and 11.91 (±6.33) dm^3 in growing season 2 (GS2). Despite high survival rates of the cuttings after planting, competitive weeds and management operations increased tree mortality during the growing season from 3.4 % up to 18.2 % averaged over the entire plantation. Weed control therefore turned out to be the key factor in the establishment success. Only a minor influence of former land use was observed during GS1, which is explained by the non-limiting nutrient conditions on both former cropland and pasture, and which disappeared during GS2. These productive soils also explained the high growth rates, with an average tree height of 247 cm and 445 cm and stem diameter (at 22 cm height) of 25.21 mm and 40.73 mm after GS1 and GS2, respectively. Genotypic and parentage variations were found to be less pronounced during GS1, and increased during GS2 as expected. The maximum leaf area index and total leaf area duration were shown to be good indicators of production and growth performance. The results of this paper confirm the high potential of SRC with poplar on agricultural land for bio-energy purposes.

1. Introduction

To meet its 20/20/20 objective, the 27 EU member states seek to reduce their CO_2 emissions by 20% and to raise the share of renewable energy by 20% by 2020 [1]. Bio-energy from biomass is one of the most interesting renewable energy sources within the EU [2]. Biomass for bio-energy can be extracted from different residue streams from agriculture, forestry and processing industries, but can also be actively grown in cultures of annual or perennial crops. For the latter, fast-growing trees are one of the most promising alternatives for the production of biomass when planted in a Short Rotation Coppice (SRC) regime. Poplar (*Populus* spp.) and willow (*Salix* spp.) are the most commonly used species for SRC in Europe [3–6]. Both species are generally propagated and grown from hardwood cuttings. Poplar is particularly suitable for SRC cultures in temperate regions because of its high growth rate and biomass yield, its easy vegetative propagation from cuttings and coppice ability, and its genetic diversity enabling growth under a wide range of environmental conditions [7,8].

* *Corresponding author*. Tel.: +32 32652827; fax: +32 32652271.
E-mail address: Laura.Broeckx@ua.ac.be (L.S. Broeckx).
[1] Both authors equally contributed to this manuscript.

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On average poplar trees in a coppice culture in temperate European conditions have dry mass yields of 10–15 Mg ha^{-1} y^{-1} [9,10]. Compared with other woody energy crops, *Populus* spp. has the advantages of a comprehensive scientific base on the ecophysiology and productivity of the genus [8,11]. SRC cultures with poplar could therefore play a promising role in achieving part of the European renewable energy objectives.

Different land use areas can be used for SRC, ranging from agricultural land, set-aside land and previously forested land to marginal land and rights-of-way of roads and highways. An SRC culture has the ability to protect the soil against erosion and nitrate leaching [12]. Some studies reported the possibility of phytoremediation of contaminated soils by SRC cultures with poplar [13,14]. Moreover, SRC plantations generally have a higher biodiversity compared to traditional agriculture [15,16].

Much information has been gathered about the cultivation, production and technological aspects of SRC cultures, leading to successful small-scale applications of SRC for bio-energy [17]. Nevertheless, the success of an SRC culture largely depends on the establishment and the first-year performance of the trees [18,19]. In general, the survival rate of dormant hardwood cuttings during the establishment year is high for commercial poplar clones. However, there is also large genotypic variation in initial rooting as affected by soil type and climatic conditions, especially soil moisture [20–22]. Cuttings are particularly prone to drought during the first weeks after planting [23]. Since poplars are light-demanding pioneer species, weed control in an SRC culture is especially critical during the period of establishment [24,8]. It is, however, unknown how a genotype may interact with former land use to affect the establishment success.

In the present study different selected poplar genotypes were assessed on establishment success and production characteristics during the first two years of an SRC culture, i.e. growing season 1 (GS1) and growing season 2 (GS2). The plantation was established on former agricultural land with two different land use types, cropland and pasture. We hypothesized that (1) genotypic variation in production characteristics will occur, quantified as stem diameter and as leaf area development, and (2) differences in production characteristics will occur on different former land use types resulting from differences in soil characteristics. The use of different genotypes in a plantation is strongly recommended to reduce the risk of large yield losses due to diseases or infestations. It is important to study the potential of these genotypes on different land areas for successful future SRC plantations. The available genotypic variation in growth characteristics can be exploited in future breeding and selection of poplar genotypes. To test these hypotheses, we established a large-scale experimental SRC culture with different poplar genotypes.

2. Materials and methods

2.1. Site description

The experimental site is located in Lochristi, Belgium (51°06′44″ N, 3°51′02″ E), about 11 km from the city of Ghent (Province East of Flanders) at an altitude of 6.25 m above sea level with flat topography. The long-term average annual and growing season temperature at the site is 9.5 °C and 13.72 °C, respectively. Average annual and growing season precipitation is 726 mm and 433 mm, respectively [25-27]. The region of the field site is pedologically described as a sandy region and has poor natural drainage [28]. The total area of the site is 18.4 ha. The former land use was (i) agriculture, consisting of cropland (ryegrass, wheat, potatoes, beets, and most recently monoculture corn with regular nitrogen (N) fertilization at a rate of 200–300 kg ha^{-1} y^{-1} as liquid animal manure and chemical fertilizers), and (ii) extensively grazed pasture.

Prior to planting, a detailed soil survey was carried out in March 2010 by analysis of soil samples taken at 110 locations, spatially distributed over the two former land use types. Bulk density and aggregate soil samples were taken at 15 cm intervals, up to 90 cm depth, by core sampling (Eijkelkamp Agrisearch equipment, Netherlands). Carbon (C) and nitrogen (N) mass fractions were determined in the laboratory by dry combustion (CN element analyzer, Carlo Erba Instruments, Italy). Soil texture, pH and nutrient concentrations were assessed on an aggregate sample of the upper 30 cm and 30–60 cm layers of the soil by the Pedological Service of Belgium (Heverlee). Based on the soil analyses, the soil type was characterized as a sandy texture with a clay-enriched deeper soil layer. The particle size distribution of mineral soil did not statistically differ among the soil layers up to 60 cm depth (Table 1).

In the upper 90 cm of the soil the average total C and N contents were not significantly different between pasture (C content of 106.0 ± 30.4 Mg ha^{-1}, N content of 9.4 ± 1.4 Mg ha^{-1}) and cropland (C content of 111.7 ± 32.9 Mg ha^{-1}, N content of 9.1 ± 2.1 Mg ha^{-1}). In the upper 0–15 cm soil layer C and N mass fractions were, however, significantly ($p = 0.000$) lower in cropland (1.48 ± 0.32% and 0.12 ± 0.03%, respectively) as

Table 1 – Soil pH, nutrient mass fractions and particle size distribution (PSD) of the soil layers at 0–30 cm and 30–60 cm depth. Weighted averages for both land use types pasture and cropland over the total land area are presented. PSD indicates that the soil has a texture of loamy sand.

			0–30 cm	30–60 cm
	pH – KCl		5.34	5.78
Nutrient mass fraction [mg kg^{-1}]	P		246.9	77.5
	K		145.4	95.1
	Mg		132.6	127.1
	Ca		1082.5	1015.5
	Na		13.3	15.2
PSD [%]	Clay	<2 μm	11.34	11.33
	Silt	2–10 μm	0.56	1.45
		10–20 μm	0.37	0.96
		20–50 μm	0.81	1.80
	Sand	>50 μm	86.93	84.45

KCl, potassium chloride; P, phosphor; K, potassium; Mg, magnesium; Ca, calcium; Na, sodium.

compared to pasture (1.95 ± 0.36% and 0.18 ± 0.03%, respectively). Soil bulk density in this upper layer was significantly higher in cropland (1.45 ± 0.07 g cm^{-3}) than in pasture (1.27 ± 0.10 g cm^{-3}). C and N mass fractions further decreased exponentially with depth (Fig. 1). The availability of nutrients K, P, Mg, Na and Ca did not differ between former land use types; averages are reported in Table 1.

2.2. Plant material and plantation establishment

A total of 14.5 ha were planted between 7 and 10 April 2010 with 12 selected poplar (*Populus*) genotypes, all commercially available. Three selected willow (*Salix*) genotypes were also planted at the same field site. The genotypes represented different species and hybrids of *Populus deltoides*, *Populus maximowiczii*, *Populus nigra*, and *Populus trichocarpa* (Table 2, [29–31]) and *Salix viminalis*, *Salix dasyclados*, *Salix alba* and *Salix schwerinii*. In this study, only results from the poplar genotypes are presented. The 12 poplar genotypes represent four different parentages.

After soil preparation by ploughing (40–70 cm depth), tilling and pre-emergent herbicide treatment, 25 cm long dormant and unrooted cuttings were planted. The cuttings were soaked in water 24 h prior to planting. The planting was performed with an agricultural leek-planting machine. The cuttings were planted in a double-row planting scheme with alternating distances of 0.75 m and 1.50 m between the rows and 1.10 m between trees within the rows, corresponding to a tree density of 8000 ha^{-1}. The plantation was designed in large monoclonal blocks of eight double rows wide (Fig. 2) that cover the two types of former land use (cropland and pasture). The minimum of two and maximum of four replicated blocks of each genotype with row lengths varying from 90 m to 340 m, were based on the available number of cuttings and the spatial configuration of the site.

During the first months after planting intensive weed control – mechanical, chemical and manual – was applied to decrease competition for light and nutrients (Table 3). With the exception of glyphosate, none of the herbicides used specify poplar as an approved crop species for the use of these chemicals. Herbicides that have proven effective in the establishment of poplar plantations in experimental trials were chosen based on the weed species present in the field (personal communication F. Goossens, ILVO), but these chemicals cannot be used legally in commercial plantations. Plantation management was extensive, without fertilization or irrigation. In the winter after GS1 the largest gaps in the plantation due to cutting mortality, were re-planted with one-year old unrooted cuttings, hereafter referred to as interplanting.

2.3. Measurements

Measurements of survival, growth performance and various production characteristics were performed on all 12 poplar genotypes. All measurements were taken during the first two years after planting between May 2010 and December 2011.

2.3.1. Leaf characteristics

Leaf area index (LAI) was assessed in different replicated measurement plots (consisting of 6 × 5 trees) for each genotype within each former land use type, i.e. two plots in GS1 and four plots in GS2 × 12 genotypes × 2 land use types. LAI was assessed using direct and indirect methods. The (cross calibrated) LAI-2000 and LAI-2200 Plant Canopy Analyzer (LiCor, Lincoln, NE, USA) was used to measure LAI indirectly in GS1 and GS2, respectively, by comparison of above and below canopy readings with a 45° view cap. These indirect LAI measurements were taken monthly throughout the growing season from July to November in GS1 and from April to November in GS2 to monitor leaf area development and to determine maximum LAI (LAI_{max}). In each plot, two diagonal transects were made between the rows, and along each transect measurements were taken with the sensor parallel

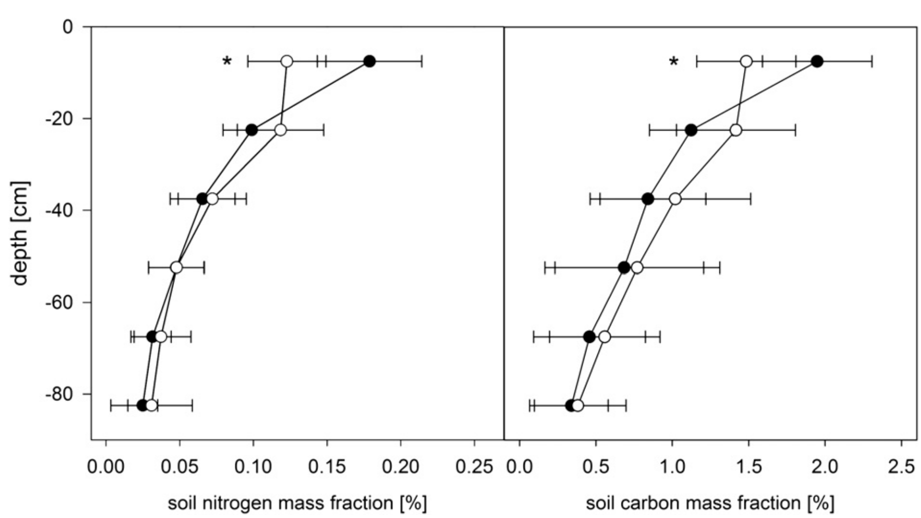

Fig. 1 – Profiles of carbon (right) and nitrogen (left) mass fractions of the soil before the plantation establishment in cropland (white circles) and pasture land (black circles). Error bars indicate standard deviation of the mean. *A significant difference between both land use types at the α = 0.05 level.

Table 2 – Overview of the twelve poplar (*Populus*) genotypes of this study planted in a Short Rotation Coppice culture. Parentage, place of origin/provenance, botanical section, year of the cross and of the commercialization, gender and selection criterion of the genotypes are shown.

Genotype	Parentage	Place of origin	Section	Year of cross/ commercialization	Gender	Selection criterion
Bakan[a]	$T \times M$	(Washington US × Oregon US) × Japan	Tacamahaca	1975/2005	♂	Plywood
Skado[a]	$T \times M$	(Washington US × Oregon US) × Japan	Tacamahaca	1975/2005	♀	Plywood
Muur[a]	$D \times N$	(Iowa US × Illinois US) × (Italy × Belgium)	Aigeiros	1978/1999	♂	Plywood
Oudenberg[a]	$D \times N$	(Iowa US × Illinois US) × (Italy × Belgium)	Aigeiros	1978/1999	♀	Plywood
Vesten[a]	$D \times N$	(Iowa US × Illinois US) × (Italy × Belgium)	Aigeiros	1978/1999	♀	Plywood
Ellert[b]	$D \times N$	Michigan US × France	Aigeiros	1969/1989	♂	Plywood
Hees[b]	$D \times N$	Michigan US × France	Aigeiros	1969/1989	♀	Plywood
Koster[b]	$D \times N$	Michigan US × The Netherlands	Aigeiros	1966/1988	♂	Plywood
Robusta[b]	$D \times N$	Eastern US × Europe	Aigeiros	1885–1890/?	♂	Plywood
Grimminge[a]	$D \times (T \times D)$	(Michigan US × Connecticut US) × (Washington US × (Iowa US × Missouri US))	Aigeiros × (Tacamahaca × Aigeiros)	1976/1999	♂	Plywood
Brandaris[b]	N	The Netherlands × Italy	Aigeiros	1964/1976	♂	Plywood
Wolterson[b]	N	The Netherlands	Aigeiros	1960/1976	♀	Plywood

D, *Populus deltoides*; *M*, *Populus maximowiczii*; *N*, *Populus nigra*; *T*, *Populus trichocarpa*.
a Produced by the Institute for Nature and Forestry Research (INBO, Geraardsbergen, Belgium).
b Produced by Vermeerderingstuinen Nederland (Zeewolde, The Netherlands).

to the row and perpendicular to the row. Leaf area duration (LAD) [m^2 day m^{-2}] was calculated as the integrated area below the seasonal LAI curve for each genotype as a function of day number of the year [20,32]. Direct LAI assessment consisted of leaf litter collection at the end of GS1 and GS2 during leaf fall, from September to December. Three 0.57 m × 0.39 m litter traps were placed on the ground along a diagonal transect between the rows in 48 plots. Litter traps were emptied every two weeks and LAI_{max} was determined using the weight method [33]. LAI_{max} was calculated from the cumulated dry weight of the leaf litter collection using a predetermined ratio between fresh leaf area and leaf dry weight, also called specific leaf area (SLA) [m^2 kg^{-1}]. Fresh leaf area was measured with a LI-3000 Leaf Area Meter (LiCor, Lincoln, NE, USA). C and N mass fractions of the leaves were determined by dry combustion (CN element analyzer, Carlo Erba Instruments, Italy) of a mixed subsample of three randomly selected mature leaves of different leaf area and on different tree heights per plot on 8 October 2010 in GS1 and on 13 September 2011 in GS2 (when LAI_{max} was reached). A weighted average LAI_{max} of the plantation was calculated based on the relative area covered by each genotype within the plantation.

2.3.2. *Stem characteristics*

Stem diameter (*D*), tree height (*H*) and mortality were measured in one entire row (between 80 and 310 trees) within each monoclonal block. *D* and *H* were assessed as the main growth characteristics after GS1, in February 2011, and repeated after GS2. Stem diameters were measured with a digital caliper (Mitutoyo, CD-15DC, UK, 0.01 mm precision) at 22 cm above soil level. This height level – recommended and used for poplar by [34,35] – is above the conical stem base and is low enough to avoid measurement errors because the stems were relatively small. A subset of a minimum of 10 stem diameters for each LAI plot was collected. If more than one stem had sprouted from one cutting, the number of stems – called shoots – was counted and diameters were measured for each shoot. The total stem basal area (BA) at 22 cm height was calculated as the sum of the basal areas of all shoots within one tree. The Huber value (HV) was calculated as the ratio between the stem basal area and the total leaf area within each plot [36]. Tree height was calculated using the predetermined relationship between *D* and *H* for each genotype, where *H* was measured with a telescopic rule (Nedo mEssfix-S, NL, 1 mm precision). Stem volume index (VI) was calculated for each genotype as the sum of $D^2 \times H$ of all shoots within one tree [34,37] and was considered as the main growth indicator of GS1 growth. Mortality was estimated by counting the number of missing trees in one or two entire rows (80–310 trees) within each monoclonal block. This was done one month after planting (May of GS1), after weed control (August of GS1) and once more in July of GS2 (after interplanting). Since genotypes Ellert, Hees and Koster had not yet fully sprouted at the time of the first mortality assessment, mortality of these genotypes has been excluded from calculation of the average plantation mortality. Mortality assessments were used to calculate effective planting density in each monoclonal block. A weighted average over the plantation was calculated for all variables, based on the relative planted area of each genotype within the plantation. The coefficient of variation was used as an indication of the variability of each production characteristic within each genotype or parentage. Production characteristics D, H, BA, VI and HV were correlated (Pearson correlation) with possible explanatory variables LAI_{max}, LAD, mortality and the number of shoots within each tree.

2.4. Statistical analysis

A nested analysis of variance (nested ANOVA) was used to analyze the influence of genotype, former land use and plot on

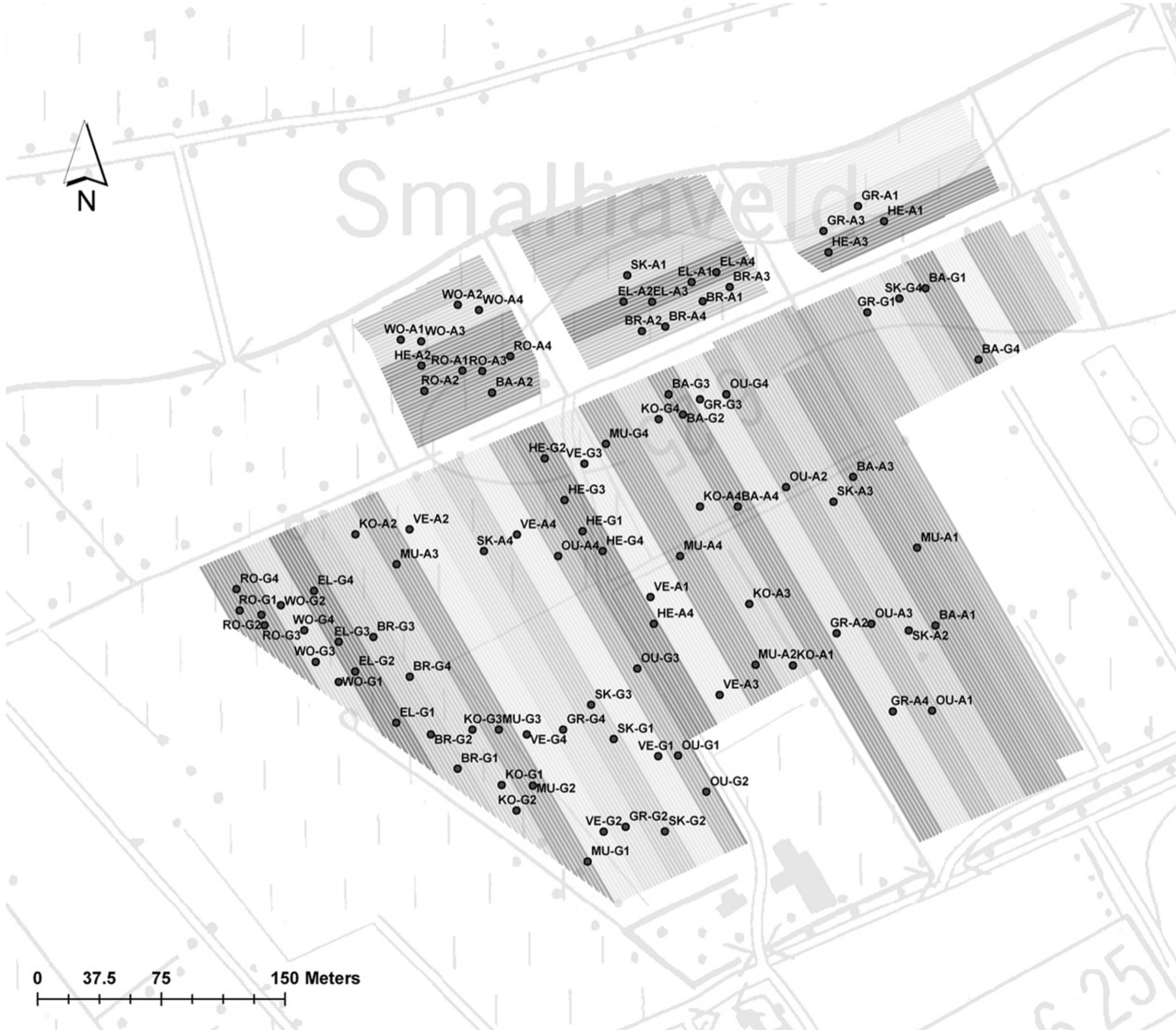

Fig. 2 – Map of the plantation lay-out showing the monoclonal blocks and the measurement plots on both former land use types. A, former cropland; G, former pasture, poplar genotypes; BA, Bakan; BR, Brandaris; EL, Ellert; GR, Grimminge; HE, Hees; KO, Koster; MU, Muur; OU, Oudenberg; RO, Robusta; SK, Skado; VE, Vesten; and WO, Wolterson.

the production characteristics described above, with (i) genotype/parentage and land use as fixed factors, and (ii) plot nested within genotype/parentage, and land use as a random factor. Data were tested for normality by means of a Kolmogorov–Smirnov test. Normal distribution was not significant in all groups and no transformation was found to normalize the data. Hence, normality was considered acceptable since no non-parametric alternative for a nested ANOVA was found [38]. When no significant land use effect or interaction between land use and genotype/parentage were found, these factors were removed from the model. The Tukey's students range test (HSD) was used as a post-hoc test in case of a significant genotype/parentage effect. LAI_{max} measurements were averaged within each plot and plots were used as replicates within each genotype × land use combination. Because of the low number of plots, a non-parametric Kruskal–Wallis test was used, followed by Mann–Whitney *U* to test each pair. A *p*-value smaller than 0.05 was considered significant. The software package SPSS (SPSS Inc., Chicago, IL, USA) was used for all analyses.

3. Results

3.1. Establishment success

Six weeks after planting initial mortality was 3.4% on average in the plantation. This number does not include genotypes Ellert, Hees and Koster which had not yet fully sprouted at the time of the mortality assessment. Mortality increased to 18.2% when weed control treatments were finished (Fig. 3). Interplanting after GS1 reduced mortality to a weighted average of 15% in the entire plantation, as assessed during GS2. A large genotypic variation in mortality was observed in the plantation, ranging between 10% and 21% after GS2. After two growing seasons genotypes Brandaris

Table 3 – Description and timing of weed control – manual, chemical and mechanical – during the establishment year (2010) and the second growing season (2011).

Category	Description	Target species	Frequency (date)	Application area	Damage on the trees
Manual					
Dutch hoe	Manual removal of weeds by pulling the hoe through the soil and cutting the weeds just under the surface	All weeds that are higher than the sprouting trees	±450 man-hours (June–August 2010)	Areas with high weed growth (i.e. weeds shading the sprouting trees)	Accidental removal of the sprout with the hoe
Chemical[a]					
Roundup[b] (glyphosate)	$3.5\ l\ ha^{-1}$ sprayed with a tractor mounted sprayer before planting	General herbicide	Single treatment (26 March 2010)	Areas with former pasture land (5.8 ha)	No assignable effect
AZ 500[c] (isoxaben) + Kerb 400 SC[2] (propyzamide)	$0.3\ l\ ha^{-1}$ AZ + $1\ l\ ha^{-1}$ Kerb sprayed with a tractor mounted sprayer before sprouting of the trees	Pre-emergent (broadleaf weeds) + soil herbicide (wide range of weeds)	Single treatment (18 April 2010)	Planted area (14.5 ha)	No assignable effect
Tomahawk[d] (fluroxypyr) + Actirob[e]	$1\ l\ ha^{-1}$ Tok + $0.5\ l\ ha^{-1}$ Acti sprayed with a tractor mounted sprayer with protective caps	Systemic herbicide (broadleaf weeds) + efficiency enhancing additive	Single treatment (24–28 June 2010)	Problem areas in terms of weed growth (6 ha)	Local tree damage by contact with the herbicide + accidental damage by tractor wheels
Matrigon[c] (clopyralid) + Actirob[e]	$1\ l\ ha^{-1}$ Mat + $0.5\ l\ ha^{-1}$ Acti sprayed with a tractor mounted sprayer with protective caps	Systemic herbicide (thistles) + efficiency enhancing additive	Single treatment (30 June–7 July 2010)	Problem areas in terms of weed (in particular thistles) growth (6 ha)	Local tree damage by contact with the herbicide + accidental damage by tractor wheels
Aramo[f] (tepraloxydim) + Actirob[e]	$2.5\ l\ ha^{-1}$ Ar + $0.5\ l\ ha^{-1}$ Acti sprayed with a tractor mounted whole field sprayer	Systemic herbicide (monocotyls) + efficiency enhancing additive	Single treatment (13 July 2010)	Problem areas in terms of weed (grasses) growth (7 ha)	Local tree damage by tractor wheels and spraying arms
Mechanical					
Tractor-pulled hoe	Weed control by agitating the soil surface in the 0.75 m and 1.50 m wide rows between the trees	Non-selective	Dual treatment (±19 May 2010 and ±3 June 2010)	Planted area (14.5 ha)	Local tree damage by tractor wheels and hoe
Mill	Milling of the soil surface with a small tractor in the 1.50 m wide rows between the trees	Non-selective	Single treatment (±25 August 2010)	Planted area (14.5 ha)	Local tree damage by tractor wheels and mill
String trimmer	Trimming the weeds in the 0.75 m and 1.50 m wide rows	Priority to thistles and weeds that are higher than 30 cm	±450 man-hours (April–September 2011)	Areas with high weed growth	Accidental removal of the tree with the trimmer
Mulcher	Mulching in the 1.50 m wide rows	Non-selective	Dual treatment (±28 May 2011 and ±8 June 2011)	Planted area (14.5 ha)	Limited damage
Heavy duty grass mower	Mowing in the 0.75 m wide rows	Non-selective	Single treatment (1–2 August 2011)	Planted area (14.5 ha)	Limited damage

a Chemical herbicides are represented by brand name, followed by the active chemical between brackets, numbers refer to the producers/distributors.
b Monsanto®.
c Dow agrosciences™.
d Agronica®.
e Bayer CropScience®.
f Certis®.

Table 4 – Results of the analysis of variance (ANOVA) of genotype, parentage, land use and plot effects on production characteristics during the first and second growing seasons.

	n	Genotype	Land use	Plot (genotype)	Parentage	Land use	Plot (parentage)
Growing season 1							
Stem diameter at 22 cm	4907	(*)	0	***	*	0	***
Basal area	4907	*	0	***	*	0	***
Number of shoots	4907	***	**	***	***	0	***
Stem height	4907	***	*	***	0	0	***
Volume index	4907	0	*	***	0	0	***
LAI_{max} indirect[a]	48	**	0	–	**	0	–
LAI_{max} direct[a]	48	*	0	–	0	0	–
Huber value indirect[a]	48	*	0	–	(*)	0	–
Huber value direct[a]	48	0	0	–	0	0	–
Growing season 2							
Stem diameter at 22 cm	4907	***	0	***	**	0	***
Basal area	4907	***	0	***	*	0	***
Number of shoots	4907	***	*	***	***	0	***
Stem height	4907	***	0	***	***	0	***
Volume index	4907	***	0	***	**	0	***
LAI_{max} indirect[a]	96	***	0	–	***	0	–
LAI_{max} direct[a]	48	**	0	–	**	0	–
Huber value indirect[a]	96	***	0	–	0	0	–
Huber value direct[a]	48	0	0	–	0	0	–

LAI_{max}, maximum leaf area index, measured (in)directly; significance: $0 = p > 0.10$; *$0.05 < p \leq 0.10$; *$0.01 < p \leq 0.05$; **$0.001 < p \leq 0.01$; ***$p \leq 0.001$; n, sample size.
a Non-parametric Kruskal–Wallis test.

and Vesten had the lowest and highest survival rates, respectively.

3.2. Production characteristics

Despite differences in soil characteristics in the upper 15 cm of the soil, only small differences were found in production related characteristics between former land use types in GS1 (Table 4). These land use differences almost disappeared in GS2, except for the number of shoots per tree.

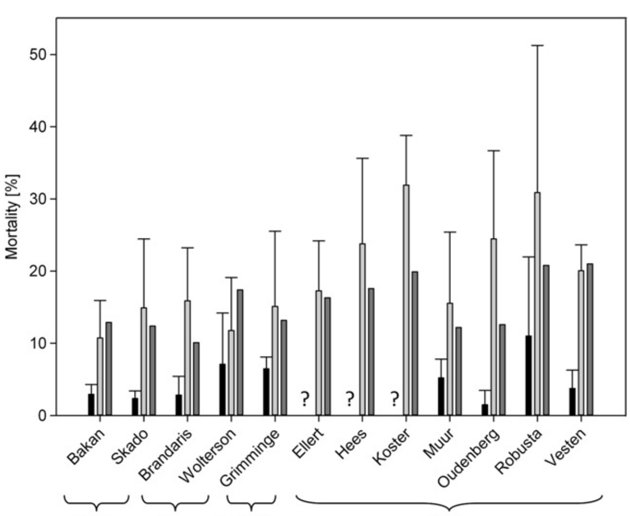

Fig. 3 – Mortality for the 12 poplar genotypes after planting (black bars), after weed control (light grey bars) during the establishment year (2010) and after interplanting (dark grey bars) at the end of the second growing season (2011). Error bars indicate standard deviation of the mean; (?) indicates cuttings had not fully sprouted at the time of mortality assessment. *D*, *Populus deltoides*; *M*, *Populus maximowiczii*; *N*, *Populus nigra*; *T*, *Populus trichocarpa*.

Weighted averages of 1.17 and 1.06 in GS1, and 2.63 and 3.07 in GS2 were found for LAI_{max} from indirect and direct measurements, respectively. Both methods were significantly correlated ($p < 0.01$ with $R^2 = 0.47$ in GS1, and $R^2 = 0.61$ in GS2). Most of the genotypes in the current study reached LAI_{max} in the beginning of October in GS1, except for genotypes Oudenberg and Robusta (early September). In GS2, LAI_{max} was reached between the end of August and mid-September. Significant genotypic differences in LAI_{max} were found in both growing seasons (Table 4), with genotype Brandaris having the lowest (0.58 ± 0.12 in GS1 and 1.05 ± 0.14 in GS2) and genotype Hees (1.87 ± 0.44 in GS1 and 4.37 ± 0.92 in GS2) having the highest LAI_{max}, measured indirectly (Fig. 4). A significant parentage effect was found for indirect measurements in GS1 and for both direct and indirect measurements in GS2. Species *P. nigra* had a significantly lower LAI_{max} compared to the hybrids in both GS1 and GS2.

The higher N mass fraction in the upper 15 cm soil layer in former pasture compared to cropland was reflected in a significantly higher leaf N mass fraction ($p = 0.009$ in GS1 and $p = 0.000$ in GS2) in trees planted on former pasture land (Fig. 5).

Stem diameter was 25.21 mm (weighted average) across genotypes at the end of GS1. Height measurements showed a weighted average of 247.4 cm (GS1) and were linearly correlated with *D*. VI was not significantly different among genotypes, although some genotypic variation was observed for stem BA and *H*. In line with LAI_{max}, a significantly lower *D* was found in *P. nigra* (genotypes Brandaris and Wolterson) in comparison to the hybrids. A low significant parentage effect

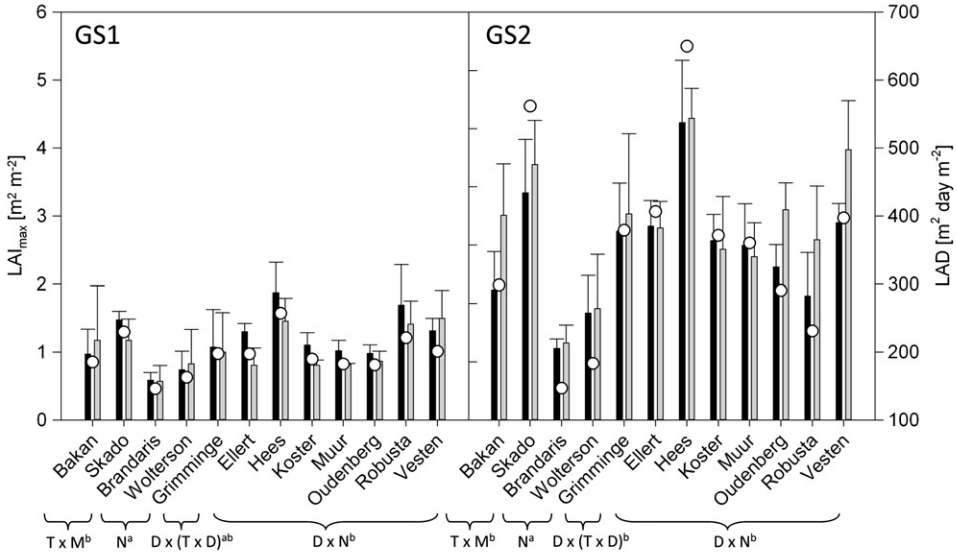

Fig. 4 – Maximum leaf area index (LAI_{max}), measured in an indirect (black bars) and direct (grey bars) way, and leaf area duration (LAD) (circles) for twelve poplar genotypes – grouped by parentage – during GS1 and GS2. [a,b]Homogeneous subsets for indirect LAI measurements, groups which do not share a letter are significantly ($p < 0.05$) different; error bars indicate standard deviation of the mean; D, *Populus deltoides*; M, *Populus maximowiczii*; N, *Populus nigra*; T, *Populus trichocarpa*; GS, growing season.

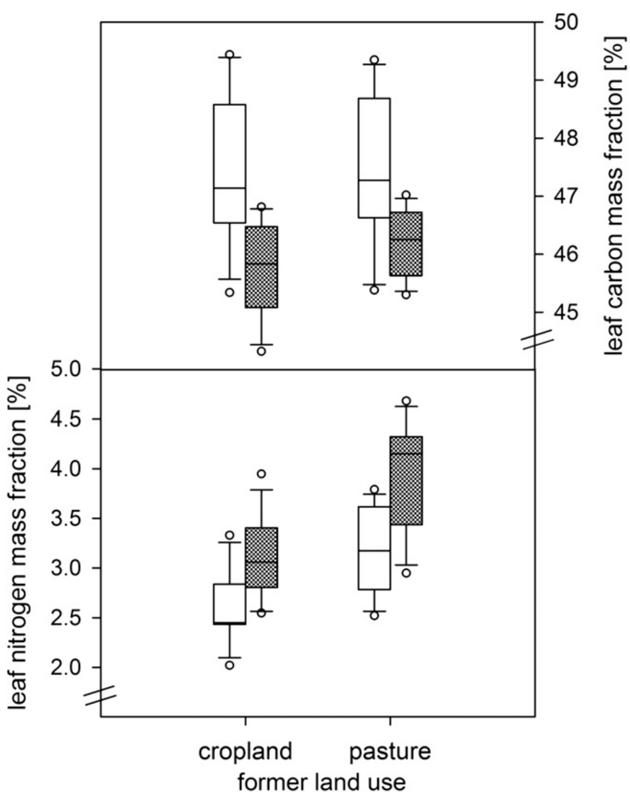

Fig. 5 – Boxplots of carbon (top) and nitrogen (bottom) mass fractions in the leaves of the different poplar genotypes (n = 12), planted on two former land use types. Plantation averages are shown. White and dotted boxes represent GS1 data and GS2 data respectively. GS, growing season.

was found for *D* and stem BA. The number of shoots that sprouted from one cutting was significantly different among genotypes and parentages. A strong plot effect within genotypes and parentages was found for all stem characteristics (Table 4), indicating a spatial variability in growth performance that was not correlated to former land use. High spatial variability was also reflected in the high variability observed for most production characteristics (Fig. 6, Table A.1), suggesting within genotype and parentage variability.

During GS2, weighted averages of 40.73 mm and 444.6 cm were found for *D* and *H*, respectively. Strong significant ($p < 0.05$) genotype and parentage effects were found for all production related characteristics (Table 4).

LAI_{max}, both from direct and indirect measurements, and LAD were strongly and linearly correlated to VI, D and BA for both growing seasons (Table 5; Fig. 7). This suggests that LAI_{max} and LAD were good indicators of production. A significant correlation with tree height was observed in GS2 as well, whereas in GS1 this was only observed for the direct LAI_{max} measurements. Lower *D* and *H* values were found with a higher number of shoots within each tree (Table 5). There was no significant correlation between mortality and VI, indicating that differences in growth performance were not caused by differences in effective planting density.

4. Discussion

An evaluation of factors affecting the first-year growth and survival is reported, since the establishment year is crucial for the success of any bio-energy plantation. Analysis of genotypic variation in production related characteristics is important to identify genotypes with high potential for

Table 5 – Correlation between production characteristics and explanatory variables (Pearson correlation).

	n	Volume index	Diameter	Basal area	Height
Growing season 1					
LAI_{max} indirect	48	++	+++	+++	0
LAI_{max} direct	48	++	+++	+++	++
LAD	48	++	+++	+++	0
No. of shoots	4907	0	−−−	+++	−−−
Mortality[a]	34	0	0	0	−
Growing season 2					
LAI_{max} indirect	96	+++	+++	+++	+++
LAI_{max} direct	48	+++	+++	+++	+++
LAD	96	+++	+++	+++	+++
No. of shoots	4907	0	−−−	+++	−−−
Mortality[a]	34	0	0	0	0

a Mortality data were collected and correlated with growth characteristics on a row level instead of a plot level, as for the other production parameters; LAI_{max}, maximum leaf area index; LAD, leaf area duration; *n*, sample size significance: $0 = p > 0.10$; $+0.01 < p \leq 0.05$; $++0.001 < p \leq 0.01$; $+++p \leq 0.001$ for positive correlations, equally with − for negative correlations.

SRC cultures, in relation to soil characteristics and former land use.

The lower C mass fraction in the upper soil layer in former cropland (Fig. 1) was caused by the annual harvesting of the crop and the removal of crop residues. A higher bulk density of the soil in cropland can be expected because of soil compaction and surface sealing by annual ploughing and regular access by agricultural machines. High uptake of N-fertilizer by the crops could explain the lower N mass fraction in the upper layer of the cropland compared to pasture, where N-rich manure remains in the field and where surplus manure coming from intensive cattle and pig farming is spread. Overall, the N mass fraction in the field site was high, but comparable with other agricultural areas in Flanders because of high N deposition (30–40 kg ha^{-1} y^{-1}) and N-fertilizer application (17–250 kg ha^{-1} y^{-1}) in this area [39]. Since N and C mass fractions in the upper soil layer (0–15 cm) were significantly higher in former pasture land, a higher growth and production could be expected on this soil type. Indeed, higher leaf N mass fractions were found in trees planted on former pasture land. However, there was only a limited effect of former land use on the measured production characteristics in GS1 and this effect disappeared during GS2. Despite significant differences, soil nutrients were clearly not limiting in either of the former land use types. Spatial variability can likely be explained by differences in weed management, since weed growth was spatially variable in the plantation. High spatial variability in mortality supports this hypothesis, since locally several adjacent trees died from mechanical and chemical treatments (Table 3). In addition, large spatial variability in the water table in the plantation could contribute to spatial variability in growth performance or indirectly to weed growth (personal communication M. Camino Serrano).

Our results confirm the high potential for the establishment and productivity of SRC with poplar on former agricultural land in terms of survival rate and growth during the first two years of the plantation. It should be noticed that these results were obtained under optimal site conditions in terms of soil quality. Survival of the planted dormant cuttings was very high, more than 96%, despite a very dry period in April 2010 (GS1) after planting (15.7 mm compared to a long-term monthly average of 51.3 mm in Ukkel, Belgium [40]). A large genotypic variation in survival rate was observed, likely driven by genotypic variation in response to the environmental conditions [22]. The delayed sprouting of genotypes Ellert, Hees and Koster compared to the other genotypes could be partly explained by a lower rooting capacity of genotypes with the *P. deltoides* parentage (female *P. deltoides* parent from the same origin; Table 2). Rooting capacity of dormant cuttings of *P. deltoides* is generally low [41] and

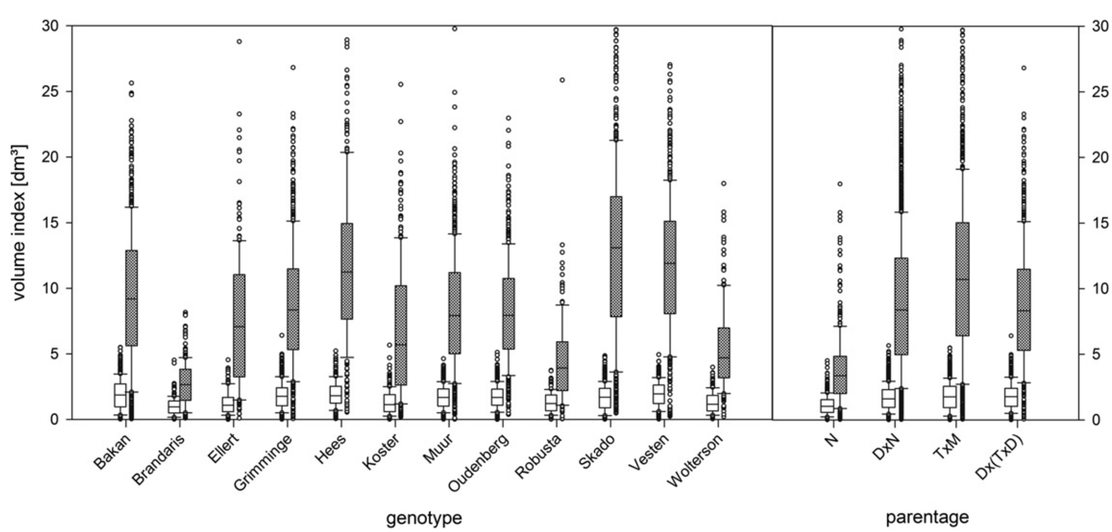

Fig. 6 – Volume index [dm^3] of trees after GS1 (white boxes) and GS2 (dotted boxes) arranged by poplar genotype (left panel) and parentage (right panel). *D*, *Populus deltoides*; *M*, *Populus maximowiczii*; *N*, *Populus nigra*; *T*, *Populus trichocarpa*; GS, growing season.

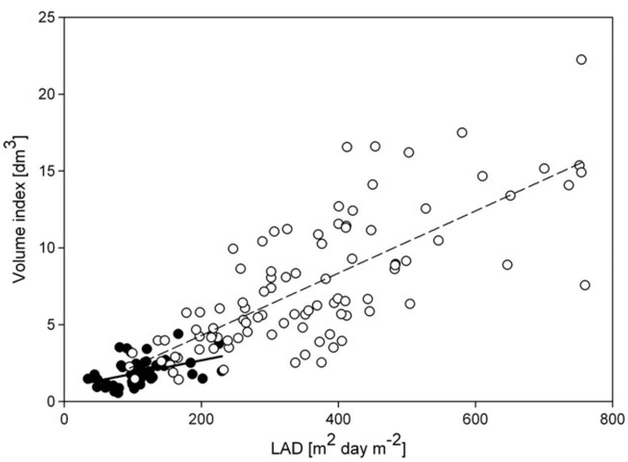

Fig. 7 – Linear correlation between leaf area duration (LAD) and volume index for 12 poplar genotypes during GS1 (black circles) and GS2 (white circles). Linear regression is significant for GS1 (solid line, $R^2 = 0.20$) and for GS2 (dotted line, $R^2 = 0.64$) at the $\alpha = 0.01$ level. GS, growing season.

genotypically highly variable [21,23]. Weed competition and weed control were the most important factors in the establishment of the plantation (cf. [24]). Poplars are known to be susceptible to competition for available water and light, mainly during the establishment year [23]. In addition, herbicide damage and losses due to mechanical weed control significantly reduced the survival rate and growth in the plantation (Table 3). Hence, genotypic variability in survival rate possibly also reflects genotypic variation in resistance to weeds or to weed control.

Even during GS2 canopy closure was not fully reached. In a comparable multi-clonal SRC plantation, stable LAI was not reached during the first year of the second rotation at a tree density of 10000 ha^{-1} [42]. The strong decrease in LAI of genotype Robusta after week 35 of GS1 can be explained by a rust infection (*Melampsora larici-populina*), an important determinant of early leaf fall [43]. No significant signs of early leaf fall or growth reduction caused by rust infection were observed for the other genotypes. During GS2, a shift in the growing season occurred; LAI_{max} was reached one month earlier as compared to GS1 due to the early growth start in 2011 from the established rooting system.

Significant genotypic variability in LAI was observed among the different poplar genotypes during GS1 and GS2, confirming previous observations [35,42,44]. From the observations of this study, genotypes with large individual leaves (Skado, Bakan and Grimminge) tended to have high LAI. Indeed, maximal individual leaf area has been reported as a main determinant of total leaf area [45,46]. However, the highest LAI was observed in genotype Hees, which can probably be explained by the fast production of many small leaves. Taylor et al. [44] reported an intermediate strategy of leaf production and individual leaf area to achieve the highest LAI. More research on the rate of leaf production and individual leaf area is required to clarify both strategies. Total leaf area determines the amount of carbon uptake and has been previously suggested as a good indicator of biomass yield of an SRC with poplar [47–49]. In this study, LAI_{max} was indeed found to be a good determinant of above-ground biomass production, assessed through VI.

In terms of D and H the first-year growth performance of the poplar genotypes in this SRC was comparable with previous reports [50–52] or higher [53,54] under similar conditions. During GS1, no significant genotypic variation was found for D or VI, although different studies reported clonal variation in biomass yield [9,50,55]. Most likely, the large spatial variability in the first-year of growth and establishment, due to the heterogeneity in weed pressure and management practices, obscured the genotypic variability in our analysis. In addition, a high variability within genotypes during the establishment year is likely due to a large heterogeneity in cutting quality, mainly depending on cutting diameter [23,25]. Indeed, large within-genotype variability was found for most production characteristics, as expressed by large variation between different measurement plots. We expect these within-genotype variations (and competition with weeds) to decrease in the following years.

GS2 data confirm an increasing genotypic variability in production characteristics, also outperforming the limited land use effects that where observed during GS1. But there was still a high spatial variability, expressed as differences among plots, probably due to growth delays of trees from lower quality cuttings and to the continuing spatially heterogeneous weed control. The parentage effect observed during GS1 was more strongly expressed in GS2. Pure *P. nigra* genotypes showed the lowest LAI and lowest diameter, indicating a significantly lower growth performance compared to the hybrid genotypes. In commercial plantations – both traditional and SRC cultures – interspecific hybrids are frequently used because of their heterosis, a phenomenon defined as the superiority of the offspring compared to the parental trees [11]. Nevertheless Al Afas et al. [54] mentioned pure *P. nigra* and *P. trichocarpa* genotypes amongst the most productive genotypes in a SRC culture at the end of the fourth growing season after coppicing. These results might indicate that the hybrids may not sustain the higher productivity over several rotations.

5. Conclusion

Only minor genotypic variation was found in production related characteristics during GS1, but some significant parentage effects were observed. Genotypic and parentage variation in growth performance were strongly expressed during GS2. Although significant differences in soil C and N mass fractions in the 0–15 cm soil layer were demonstrated between former cropland and pasture, this was reflected only to a small extent in different growth performance of the trees planted on both former land use types. This former land use effect was clearly outperformed by the genotypic variation during the second growing season. High spatial variability in production characteristics among the different genotypes was likely influenced by weed management, which turned out to be the key factor in the establishment success of an SRC plantation.

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Appendix A

Table A.1 – Production characteristics for 12 poplar genotypes after growing season 1 (GS1) and growing season 2 (GS2), averaged by genotype, by relative area in the plantation and by parentage. Huber value ((*in*)direct) = stem basal area/leaf area using (*in*)direct assessment.

	n	Stem diameter at 22 cm [mm]	Stem basal area at 22 cm [mm^2]	Tree height [cm]	Number of shoots	Volume index [cm^3]	*n*	Huber value (indirect) [10^{-4} m^2/m^2]		Huber value (direct) [10^{-4} m^2/m^2]
GS1										
Bakan	668	24.43 (30)	513.2 (49)^{bc}	262.7 (21)^{g}	1.02 (18)^{a}	1885 (61)	4	4.56 (20)^{cd}		4.36 (50)
Brandaris	231	19.08 (33)	343.5 (58)^{a}	213.6 (17)^{c}	1.23 (40)^{b}	1004 (68)	4	5.18 (35)^{bcd}		5.87 (50)
Ellert	201	20.79 (34)	466.3 (60)^{bc}	159.7 (21)^{a}	1.49 (41)^{d}	1238 (74)	4	2.89 (15)^{a}		4.79 (13)
Grimminge	612	24.79 (49)	523.2 (46)^{cd}	254.8 (18)^{fg}	1.03 (21)^{a}	1824 (58)	4	4.16 (30)^{bcd}		4.45 (22)
Hees	327	26.19 (29)	710.8 (46)^{e}	205.5 (12)^{abc}	1.46 (45)^{cd}	1907 (51)	4	3.02 (6)^{ab}		4.00 (28)
Koster	284	21.54 (20)	455.8 (24)^{b}	210.6 (9)^{bc}	1.26 (45)^{b}	1302 (22)	4	2.76 (17)^{a}		3.78 (22)
Muur	652	23.97 (26)	520.4 (38)^{cd}	243.0 (14)^{de}	1.20 (39)^{b}	1682 (43)	4	3.87 (36)^{abcd}		4.62 (22)
Oudenberg	529	23.95 (24)	514.9 (37)^{bc}	253.2 (13)^{f}	1.21 (49)^{b}	1727 (52)	4	5.44 (19)^{d}		6.09 (8)
Robusta	137	22.75 (31)	476.8 (50)^{bc}	202.9 (18)^{ab}	1.23 (43)^{b}	1318 (62)	4	2.69 (14)^{a}		3.13 (15)
Skado	601	23.90 (30)	495.0 (49)^{bc}	246.5 (18)^{def}	1.04 (22)^{a}	1684 (59)	4	3.28 (14)^{ab}		4.34 (34)
Vesten	515	26.07 (24)	577.7 (41)^{d}	248.6 (16)^{ef}	1.05 (23)^{b}	1931 (50)	4	3.66 (20)^{abc}		3.26 (7)
Wolterson	164	20.36 (31)	392.4 (49)^{a}	239.7 (19)^{d}	1.38 (53)^{c}	1278 (63)	4	4.70 (54)^{abcd}		5.78 (89)
Weighted average		23.79	513.1	240.3	1.16	1671		4.51		5.21
N	395	19.61 (32)^{a}	363.8 (54)^{a}	224.4 (19)	1.29 (47)^{c}	1118 (67)	8	4.94 (42)		5.82 (67)
D × *N*	2645	24.08 (27)^{b}	540.7 (49)^{c}	232.1 (18)	1.24 (42)^{b}	1374 (57)	28	3.47 (33)		4.24 (28)
T × *M*	1269	24.17 (30)^{b}	504.6 (49)^{b}	255.0 (20)	1.03 (20)^{a}	1790 (61)	8	3.92 (24)		4.35 (40)
D × (*T* × *D*)	612	24.79 (49)^{b}	523.2 (46)^{bc}	254.8 (18)	1.03 (21)^{a}	1824 (58)	4	4.16 (30)		4.45 (22)
GS2										
Bakan	668	40.33 (26)^{cd}	1370.0 (45)^{c}	496.7 (20)^{i}	1.03 (19)^{a}	9408 (56)^{e}	8	5.39 (23)^{de}	4	3.33 (14)
Brandaris	231	27.58 (29)^{a}	694.4 (51)^{a}	290.4 (19)^{a}	1.23 (39)^{b}	2746 (62)^{a}	8	4.84 (23)^{cde}	4	4.56 (42)
Ellert	201	37.76 (29)^{c}	1476.2 (54)^{c}	372.6 (23)^{d}	1.55 (41)^{d}	7461 (69)^{cd}	8	3.75 (19)^{ab}	4	3.95 (11)
Grimminge	612	41.25 (23)^{d}	1426.2 (42)^{c}	447.1 (18)^{fg}	1.04 (19)^{a}	8673 (54)^{de}	8	4.03 (20)^{abc}	4	3.55 (29)
Hees	327	47.07 (24)^{e}	2241.5 (45)^{e}	406.0 (13)^{e}	1.49 (47)^{cd}	11914 (53)^{f}	8	4.46 (19)^{bcd}	4	3.83 (17)
Koster	284	38.10 (15)^{c}	1408.7 (19)^{c}	344.7 (9)^{c}	1.28 (45)^{b}	6806 (14)^{c}	8	3.49 (20)^{a}	4	3.95 (14)
Muur	652	40.21 (23)^{cd}	1426.9 (32)^{c}	434.6 (13)^{f}	1.22 (39)^{b}	8280 (54)^{de}	8	4.34 (17)^{bcd}	4	4.16 (9)
Oudenberg	529	39.53 (21)^{cd}	1362.6 (28)^{c}	460.1 (10)^{gh}	1.24 (55)^{b}	8244 (48)^{de}	8	4.97 (31)^{abcdef}	4	3.82 (19)
Robusta	137	33.11 (32)^{b}	1010.1 (55)^{b}	321.3 (20)^{b}	1.22 (43)^{b}	4518 (75)^{b}	8	6.49 (21)^{f}	4	4.30 (14)
Skado	601	45.03 (25)^{e}	1710.7 (44)^{d}	556.4 (16)^{j}	1.05 (27)^{a}	12937 (55)^{f}	8	3.85 (28)^{abc}	4	4.38 (20)
Vesten	515	46.77 (23)^{e}	1835.7 (38)^{d}	476.9 (14)^{h}	1.06 (25)^{a}	11680 (47)^{f}	8	5.12 (16)^{de}	4	3.99 (14)
Wolterson	164	34.35 (26)^{b}	1082.9 (43)^{b}	371.1 (19)^{d}	1.41 (54)^{c}	5405 (62)^{b}	8	6.20 (38)^{ef}	4	5.74 (23)
Weighted average		40.73	1483.9	444.6	1.18	9053		5.44		4.71
N	395	30.39 (30)^{a}	855.7 (53)^{a}	323.9 (23)^{a}	1.30 (47)^{b}	3850 (74)^{a}	16	5.52 (35)	8	5.15 (32)
D × *N*	2645	41.42 (27)^{b}	1574.6 (50)^{c}	424.2 (20)^{b}	1.26 (44)^{b}	8969 (60)^{b}	56	4.66 (29)	28	4.00 (13)
T × *M*	1269	42.56 (26)^{b}	1531.3 (46)^{b}	525.0 (19)^{d}	1.04 (23)^{a}	11079 (59)^{c}	16	4.62 (30)	8	3.85 (22)
D × (*T* × *D*)	612	41.25 (23)^{b}	1426.2 (42)^{bc}	447.1 (18)^{c}	1.04 (19)^{a}	8673 (54)^{b}	8	4.03 (20)	4	3.55 (29)

Numbers between brackets indicate coefficient of variation [%]; a, b, c, d homogeneous subsets, groups which do not share a letter are significantly ($p < 0.05$) different; *n*, sample size; *D*, *Populus deltoides*; *M*, *Populus maximowiczii*; *N*, *Populus nigra*; *T*, *Populus trichocarpa*.

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